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Commutability of proficiency testing material containing tobramycin: a study within the framework of the Dutch Calibration 2.000 project

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Abstract

Background: Results from external quality assessment schemes (EQASs) can provide information about accuracy and comparability of different measurement methods, provided that the material used in these schemes behave identical to patient samples among the different methods, a characteristic also known as commutability. The aim of

this study was to assess the commutability of different matrices for the material used in an EQAS for tobramycin.

Methods: Proficiency testing material (PTM) and patient samples containing tobramycin were prepared, collected, pooled, and distributed to participating laboratories for analysis. Low, medium, and high tobramycin concentrations in liquid human, liquid bovine and lyophilized bovine serum were tested in this study. The patient serum results of every laboratory were plotted against each of the other laboratories, and the distances of the PTM results to the patient serum regression line were calculated. For comparison, these distances were divided by the average within-laboratory standard deviation (SD_{wl}) of the results reported in the official EQAS for tobramycin, resulting in a relative residual. The commutability decision limit was set at $3 SD_{wl}$.

Results: With 10 laboratories participating in this study, 45 laboratory couples were formed. For human serum, only one relative residual for high concentrations of tobramycin was found outside the commutability decision limit. For liquid and lyophilized bovine sera, the number of relative residuals outside the decision limit was between 15 and 18 for low, medium, and high tobramycin concentrations.

Conclusions: The PTM used for tobramycin is preferably prepared with human serum.

Keywords: commutability; external quality assessment scheme; immunoassay; proficiency testing.

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Introduction

Results from external quality assessment schemes (EQASs) can serve multiple purposes [1]. Foremost, the participating individual laboratory can compare its own results to the target value and/or to the results of the other participating laboratories, indicating good, acceptable, or poor performance in that particular analysis. A second outcome of the results of EQAS is the information about the accuracy and

comparability of different measurement methods. Every medical laboratory is obliged to perform in these inter-laboratory comparisons, according to the International Organization for Standardization (ISO) standard 15189 [2].

For genuine comparison of results within and between laboratories and methods, the samples used in EQAS preferably behave identical to patient samples among the different methods of analysis used [3–6]. This characteristic is called commutability and is defined by the Clinical and Laboratory Standards Institute (CLSI) as “the ability of a material to yield the same numerical relationships between results of measurements by a given set of measurement procedures, purporting to measure the same quantity, as those between the expectations of the relationships obtained when the same procedures are applied to other relevant types of material” [7].

After commutability has been established for a material used in EQAS, the unbiased comparison of results between laboratories and methods can reveal the variation among methods and serve as a starting point for harmonization [8], which is of particular importance given the fact that hospitals specialize in different treatments and patients visit more than one hospital. With harmonization, patient care can be improved, clinical guidelines can be better applied, and the amount of medical errors and healthcare costs can be decreased [8].

The Dutch EQAS organizer, the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML), provides inter-laboratory comparisons which cover the entire field of medical laboratories. The Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology (KKGt), is a section of SKML and provides the inter-laboratory comparisons in the field of therapeutic drug monitoring and clinical toxicology. This commutability study is the second commutability study performed by KKGt and is part of the Dutch project “Calibration 2.000” [9]. The first study examined the commutability of the sample for analysis of anti-epileptic drugs [10]. Carbamazepine and valproic acid were chosen to represent the sample containing a total of nine anti-epileptic drugs. In this study, the antibiotic drugs sample is evaluated for commutability.

Materials and methods

Study design

The study design of this commutability evaluation study is comparable to the design of the commutability evaluation of proficiency

testing material (PTM) for carbamazepine and valproic acid [10]. In this previous commutability study with carbamazepine and valproic acid, in which carbamazepine and valproic acid were the least and the most robust process, respectively, no difference in commutability could be demonstrated between the most and least robust process [10].

In the antibiotic drugs PTM, tobramycin was the least robust proficiency testing (PT) process according to the retrospective data analysis of the averages of results reported by all participants over a period of 10 years. The process capability index (C_{pk}) [11] values were 0.24, 0.32, 0.37, 0.17, and 0.63 for amikacin, gentamicin, methotrexate, tobramycin, and vancomycin, respectively. Since no difference in commutability could be demonstrated in the aforementioned carbamazepine and valproic acid study [10] between the most and least robust process, only tobramycin was chosen to be tested in this study because we assume that tobramycin is the most prone to non-commutability since the results of the analysis in our EQAS is the least robust.

Candidate matrices

In this commutability study, two different origins of matrices are tested, human and bovine serum. The samples are preferably distributed to the participants by mail, therefore the effect of lyophilization of bovine serum on commutability was also tested. A total of three candidate matrices are tested in this study: frozen liquid human serum, frozen liquid bovine serum, and lyophilized bovine serum.

Blank bovine serum was purchased from Invitrogen (Paisley, Scotland, UK, www.invitrogen.com) in 500-mL bottles of gamma irradiated newborn calf serum. This serum was stored at -20°C and slowly defrosted overnight at room temperature before sample preparation. Blank human serum was obtained from healthy adults who participated in a hepatitis screening program in the first-line treatment. The sera of approximately 70 healthy adults were collected in laboratory tubes and stored at -20°C within 1 month before sample preparation and were defrosted at room temperature for 2 h before pooling. Pooling was performed on an open laboratory bench, where no protection for bacterial exposure was used and materials were not filtered before dispensing. The serum pool was tested for the presence of tobramycin and other drugs, and the presence of HIV-1/2 antibodies, hepatitis B surface antigen, and hepatitis C virus antibody. All test results were negative.

Both blank matrices were used for preparation of the candidate matrices samples.

Candidate matrices sample preparation

The blank candidate matrix samples were prepared by adding a volumetric quantity of a tobramycin stock solution in water to both matrices. 110 mL blank bovine serum was spiked with 2.0, 4.0, and 6.0 mL of a tobramycin stock solution, and 5.00 mL blank human serum was spiked with 100, 165, and 265 μL tobramycin stock solution, to obtain low, medium, and high concentrations tobramycin (see Table 1). Matrices were stirred for 10 min, according to the local preparation protocol for EQAS material. The bovine serum was dispensed in vials in 2-mL aliquots, and half of the batch was lyophilized. The human serum was dispensed in laboratory vials in 250- μL aliquots. Both

Table 1: Tobramycin concentrations (in mg/L) in candidate matrices samples.

	Human serum (liquid)	Bovine serum (liquid)	Bovine serum (lyophilized)
Low	2.9	2.7	2.6
Medium	4.8	5.4	5.2
High	7.5	8.2	7.8

liquid bovine serum and liquid human serum samples were stored at -20°C and lyophilized bovine serum samples were stored in the refrigerator prior to dispatch.

Patient material preparation

Patient serum samples containing tobramycin left over from clinical analysis were collected during 3 months prior to the study. Hemolytic and icteric samples were rejected, no criteria for lipemic samples were defined. Samples were stored at -80°C and defrosted at room temperature for 1 h prior to pool preparation. Pooling was performed on an open laboratory bench, where no protection for bacterial exposure was used and materials were not filtered before dispensing. After pool preparation and partition in aliquots, the samples were stored at -20°C prior to dispatch.

Six pool sera, including one blank serum, were prepared from the leftover patient serum samples, resulting in tobramycin concentrations covering the range normally covered by immunoassays. The tobramycin concentrations of the pool sera were 1.8, 3.8, 5.5, 8.0, and 9.3 mg/L, determined as the mean value of the results reported by the laboratories participating in this commutability study.

Participants and measurement methods

Participants of the Dutch antibiotic drugs EQAS were asked to perform in this commutability study. Among the participants, a selection was made according to the immunoassay in use, to include every immunoassay currently used for the analysis of tobramycin.

Written instructions about dispatch, storage condition, reconstitution of the lyophilized samples, and minimum/maximum time between samples receipt and analysis were sent to the participating laboratories. All materials were sent on dry ice and delivered within 24 h to the laboratories. Participants were instructed to analyze the samples on the day of receipt or within 24 h. Liquid samples which were received in a frozen state could be stored at -20°C prior to analysis, but needed to be analyzed within 24 h. Frozen samples were thawed at room temperature. Liquid samples which were thawed at arrival needed to be analyzed immediately or stored in the refrigerator and analyzed within 24 h. Participants were instructed to homogenize the thawed liquid samples, a vortex could be used. Lyophilized samples needed to be restored in the refrigerator until reconstitution and analysis. Participants were instructed to reconstitute these samples by adding 2.00 mL of distilled water, leave the vial for 15 min on the workbench, and then carefully mix without shaking until dissolved completely. Every sample needed to be analyzed in duplicate in a single run to improve statistical power.

Ten laboratories participated in this commutability evaluation. All laboratories used an immunoassay method for the analysis of tobramycin. One laboratory was not able to analyze the samples in duplicate, and a second laboratory stated no lyophilized bovine serum samples were received.

Five different immunoassay methods were used. Four laboratories used the fluorescence polarization immunoassay (FPIA) method by Abbott, two used FPIA by Roche, two used cloned enzyme donor immunoassay (CEDIA) by Roche, one used enzyme multiplied immunoassay technique (EMIT) by Siemens, and one used particle-enhanced turbidimetric inhibition immunoassay (PETINIA) by Siemens. With 10 laboratories participating in this commutability evaluation, 45 laboratory couples could be formed with 13 different method comparisons (see Table 2).

Data analysis

Data analysis was performed comparable to the data analysis in the carbamazepine and valproic acid commutability evaluation [10]. CLSI guideline EP30-A [12] was applied for data analysis.

In brief, the results of every laboratory were compared to the results of each of the other laboratories. The mathematical relationships of the tobramycin results of the patient samples between the laboratories were compared to the mathematical relationships of the results of the candidate matrix samples (see Figure 1 for an example, see Supplemental Figure 1 for results from all 45 laboratory couples).

To express commutability, the orthogonal residuals between the results of the candidate matrix samples and the patient samples using Passing and Bablok regression are calculated and for comparison expressed as a multiple of the within-laboratory standard deviation (SD_{wl}). This SD_{wl} is the average SD_{wl} calculated from EQAS results for tobramycin over a period of 3 years. The commutability decision limit was set at 3SD_{wl} , which is a more robust, consistent, and mostly more stringent limit than two standard error of regression of each pair of laboratories.

Table 2: Number of laboratory couples for each method comparison.

Method 1	Method 2	Number of method comparisons
FPIA Abbott	FPIA Abbott	6
	FPIA Roche	8
	CEDIA Roche	8
	EMIT Siemens	4
	PETINIA Siemens	4
FPIA Roche	FPIA Roche	1
	CEDIA Roche	4
	EMIT Siemens	2
	PETINIA Siemens	2
CEDIA Roche	CEDIA Roche	1
	EMIT Siemens	2
	PETINIA Siemens	2
EMIT Siemens	PETINIA Siemens	1
Total		45

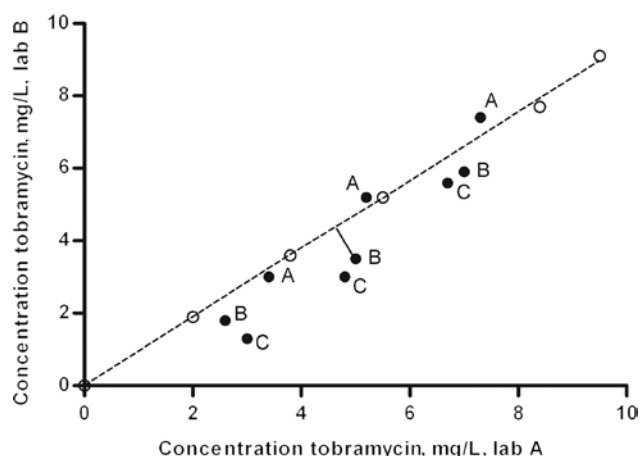


Figure 1: Example of data analysis of two laboratories. O, patient samples; ●, candidate matrix samples (A, B, or C); dashed line (---), patient sample regression line; continuous line (—), orthogonal residual.

Results

Calculated relative residuals are presented in Figure 2. The analysis of tobramycin in liquid human serum produced only one relative residual outside the commutability cut-off limit of $3 SD_{wl}$, for the highest concentration. This relative residual was produced by a combination of laboratories which both used the FPIA method by Abbott. For the analysis of all other samples by these two laboratories, no relative residuals outside the commutability cut-off limit were produced.

For the liquid and lyophilized bovine serum samples, a large amount of relative residuals were outside the

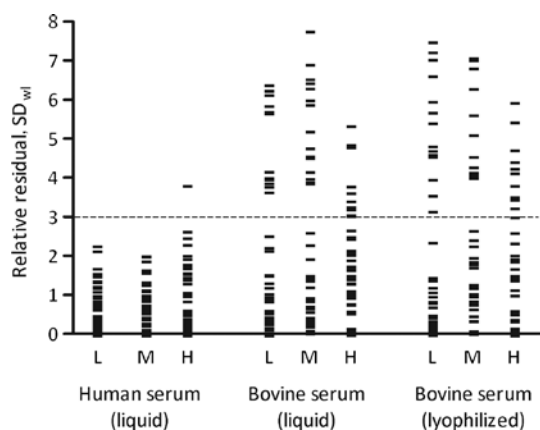


Figure 2: Relative residuals for liquid human, liquid bovine and lyophilized bovine sera spiked with low (L), medium (M) and high (H) concentrations of tobramycin. Commutability decision limit at $3 SD_{wl}$, indicated by dashed line (---).

commutability cut-off value. For liquid bovine serum 15, 15, and 11 relative residuals exceeded $3 SD_{wl}$, for low, medium, and high concentrations of tobramycin, respectively. For lyophilized bovine serum these numbers were 14, 12, and 12 for low, medium, and high concentrations, respectively.

Every laboratory couple with a CEDIA method by Roche produced relative residuals outside $3 SD_{wl}$ for liquid and lyophilized bovine serum. The laboratory couple consisting of the two CEDIA methods by Roche did not produce relative residuals outside $3 SD_{wl}$ for liquid and lyophilized bovine serum.

When an even more strict commutability decision limit of $2 SD_{wl}$ had been chosen, the result remains the same. The number of relative residuals is the lowest in the liquid human serum samples and increases in both liquid and lyophilized bovine serum samples (see Table 3).

Discussion

The current commutability study is designed comparable to the previous carbamazepine and valproic acid commutability study [10]. Due to an expensive and time-consuming commutability assessment method described in CLSI EP30-A guideline [12], both studies were designed as an X-ring design, in which every laboratory is coupled with each of the other laboratories, thereby creating multiple method comparisons. Because of this design, every laboratory needed to receive the same sera; therefore, large amounts of patient sera in different concentrations were required, for which patient sera were pooled. According to CLSI EP30-A, the commutability of this pooled serum needs to be examined, which is even more work than this commutability study. Therefore, a small experiment was conducted to assess the deviation of the measured tobramycin concentration in the pool vs. the calculated tobramycin concentrations on the basis of the individual tobramycin concentrations. The deviations were 10%, 2%, -4%, 5%, and -2% for the respective pooled patient materials from the lowest to the highest concentration.

This pooling of sera can be an advantage due to diluting interfering substances, which can confound results of the commutability study, but can also be a disadvantage if one of the patient samples contains an interfering substance which contaminates the entire, thereby confounding the results. In this commutability study, approximately 10 patient sera were used for preparation of each of the serum pools; therefore, a potential interfering substance will probably be adequately diluted.

Table 3: Number of relative residuals for the analysis of tobramycin above the commutability decision limit at 2 SD_{wl} and 3 SD_{wl} , respectively.

		Human serum (liquid)		Bovine serum (liquid)		Bovine serum (lyophilized)	
		2 SD_{wl}	3 SD_{wl}	2 SD_{wl}	3 SD_{wl}	2 SD_{wl}	3 SD_{wl}
Tobramycin	Low	2	0	18	15	15	14
	Medium	2	0	17	15	15	12
	High	5	1	18	11	15	12

This pooling could be indicated as a shortcoming of the commutability study because preferably only individual patient sera including interfering substances would be used to represent the patient sample population. On the other hand, in an EQAS, the used samples will primarily be serum samples without interfering substances, to represent the majority of the patient sample population.

Results from this study indicate that material made with human serum behaves more like patient samples compared to bovine serum (both liquid and lyophilized), indicating that PTM is preferably prepared with human serum.

All relative residuals outside 3 SD_{wl} for the analysis of tobramycin in liquid and lyophilized bovine serum samples were produced by every laboratory couple containing one CEDIA method by Roche. Relative residuals produced by the combination of two laboratories who both use the CEDIA method were 2.17, 2.32, and 1.54 for low, medium, and high concentrations of tobramycin, respectively in liquid bovine serum, and 2.39, 2.45, and 1.17 for low, medium, and high concentrations of tobramycin, respectively, in lyophilized bovine serum. In human serum, no relative residuals for low, medium, and high tobramycin concentrations exceeded 3 SD_{wl} and only one relative residual exceeded 2 SD_{wl} (2.05) for laboratory couples containing one CEDIA method, indicating the CEDIA method is sensitive for components in the bovine serum matrices. This CEDIA method is no longer available for the analysis of tobramycin.

The one relative residual for human serum outside the commutability cut-off value of 3 SD_{wl} is produced by two laboratories who both use the Abbott FPIA method. Other laboratory couples with a combination of two Abbott FPIA methods produced relative residuals for the highest concentration of tobramycin in human serum of 0.88, 1.61, 1.74, 2.67, and $2.50\text{ SD}_{\text{wl}}$. All but one of the nine relative residuals outside 2 SD_{wl} in human serum were produced by laboratory couples of which at least one of the laboratories used the FPIA method by Abbott. This indicates that the candidate human serum sample might potentially be unsuitable for the analysis of high concentrations

of tobramycin. The FPIA method of Abbott is no longer available.

The relative residuals outside 3 SD_{wl} in the carbamazepine commutability study might be the result of cross-reactivity of the carbamazepine-epoxide metabolite in the immunoassays, but this cross-reactivity cannot explain the deviating relative residual for tobramycin in human serum since tobramycin is excreted unchanged [13]. Evaluation studies regarding the comparability between different immunoassays show significantly different results between methods [14, 15]. This possibly contributes to the relative residuals outside commutability decision limit.

A shortcoming of this study is the absence of a tobramycin sample in lyophilized human serum. Due to shipment of the samples in EQAS with regular mail, a lyophilized sample is preferred for transportation. However, a lyophilized sample has to be reconstituted, which carries an additional variability and patient samples are not lyophilized. Even though relative residuals for liquid and lyophilized bovine serum are comparable, these results cannot be extrapolated from liquid to lyophilized human serum.

Conclusions

Even though not all relative residuals for the analysis of tobramycin in human serum are below the commutability cut off limit of 3 SD_{wl} , human serum is the preferred matrix for tobramycin in external quality assessment.

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